GLUFOSINATE-AMMONIUM
Shaughnessy No. 128850
(DP Barcodes D238707 and D246288)

Permanent Tolerance Petition (PP#7F04910) For Use Of Glufosinate-Ammonium On Transgenic Canola And Sugar Beets

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Submitted by:
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GLUFOSINATE-AMMONIUM

PERMANENT TOLERANCE PETITION (PP#7F04910) FOR USE OF GLUFOSINATE-AMMONIUM ON TRANSGENIC CANOLA AND SUGAR BEETS (DP BARCODES D238707 AND D246288)

INTRODUCTION

AgrEvo USA Company has submitted metabolism studies with canola (1993; MRID 44358606; and 1994; MRID 44358607) and sugar beets (1996; MRID 44358601) in support of a petition for the establishment of permanent tolerances for residues of the non-selective herbicide glufosinate-ammonium. AgrEvo is also requesting an amendment to the Section 3 registration of the 1.67 lb/gal soluble concentrate formulation (Liberty™ Herbicide, EPA Reg. No. 45639-199) to add use of glufosinate-ammonium on canola and sugar beets which have been genetically modified to be tolerant to glufosinate-ammonium. The petitioner is proposing the establishment of permanent tolerances for residues of glufosinate-ammonium and its metabolites 2-acetamido-4-methylphosphinico-butanoic acid and 3-methylphosphinico-propionic acid expressed as glufosinate free acid equivalents in/on the following commodities derived from transgenic canola and sugar beets that are tolerant to the herbicide glufosinate-ammonium:

Beet, sugar, root	0.7 ppm
Beet, sugar, tops (leaves)	1.3 ppm
Beets, sugar, molasses	5.0 ppm
Canola seed	0.4 ppm
Canola meal	2.0 ppm

Glufosinate-ammonium [ammonium-DL-homoalanin-4-yl-(methyl)phosphinate] is an herbicide currently registered for both non-selective and selective uses. Selective uses involve application to plants that have been genetically transformed to be resistant to the herbicide. Transgenic plants contain a gene which enables the plant to rapidly metabolize the herbicidally active moiety of glufosinate-ammonium into a metabolite (2-acetamido-4-methylphosphinico-butanoic acid, also termed N-acetyl-glufosinate) which is not herbicidally active and which is not found in non-transgenic plants. Glufosinate-ammonium is a racemic mixture of the D- and L-isomers; only the L-isomer is herbicidally active.

Time-limited tolerances have been established for residues of glufosinate-ammonium and its metabolite, 3-methylphosphinico-propionic acid, in/on almond hulls, apples, grapes, the tree nuts group, eggs, milk, and the fat, meat, and meat byproducts of cattle, goats, hogs, horses, poultry, and sheep; the tolerances have an expiration date of July 13, 1999 [40 CFR §180.473(a)]. An import tolerance with an expiration date of January 18, 2000 has been established for combined residues of glufosinate-ammonium and its metabolite, 3-methylphosphinico-propionic acid, expressed as glufosinate acid equivalents, in/on bananas [40 CFR §180.473(b)]. In addition, time-limited tolerances, with an expiration date of July 13, 1999, have been established for residues of glufosinate-ammonium and its metabolites, 2-acetamido-4-methylphosphinico-butanoic acid and 3-methylphosphinico-propionic acid, in/on aspirated grain fractions, field corn grain, forage, and stover, soybeans, and soybean hulls derived from transgenic corn and soybeans that are tolerant to glufosinate ammonium [40 CFR §180.473(c)].

CONCLUSIONS AND RECOMMENDATIONS

OPPTS GLN 860.1300: Nature of the Residue - Plants

1a. Sugar beets: The qualitative nature of the residue in transgenic sugar beets is adequately understood. Total radioactive residues (TRR) were 2.05 ppm in tops and 0.93 ppm in roots harvested 146 days following two applications of [^{14}C]glufosinate-ammonium at 0.54 lb ai/A (total application rate of 1.07 lb ai/A; 1x the maximum proposed seasonal rate). Samples of sugar beet commodities were also collected at shorter posttreatment intervals (PTIs); TRR were 20.08 ppm in tops and 2.01 ppm in roots collected at a 0-day PTI and were 12.26 ppm in tops and 6.75 ppm in roots collected at a 21-day PTI.

1b. In sugar beet tops and roots, ~93-98% of TRR was identified. The N-acetyl-glufosinate metabolite was the major residue in all commodities at all PTIs, except tops at the 0-day PTI, accounting for 55.2-67.9% of TRR (0.63-6.77 ppm). Glufosinate-ammonium accounted for 84.6% of TRR (16.97 ppm) in 0-day PTI tops, and accounted for 19.1-41.8% of TRR (0.18-5.12 ppm) in sugar beet commodities at other PTIs. 3-Methylphosphinico-propionic acid (MP-propionic acid) was identified at low levels in all sugar beet commodities at all PTIs (0.4-6.0% TRR, 0.04-0.14 ppm). One additional metabolite, 2-methylphosphinico-acetic acid, was identified in 146-DAT tops at 0.07% TRR (0.001 ppm).

2a. Canola: The submitted study is marginally adequate to delineate the nature of the residue in transgenic canola. The test substance was applied at less than 1x the maximum proposed seasonal rate which resulted in low levels of radioactivity in canola seed, making identification of residues difficult. However, because adequate metabolism studies with transgenic field corn and soybeans have been submitted previously.(PP#5G4466 and PP#5F4578; CB Nos. 15081 and 16154, DP Barcodes D211531 and D219069, 3/7/96,

M. Rodriguez), no additional data pertaining to the metabolism of glufosinate-ammonium in canola will be required.

2b. Total radioactive residues (TRR) were 0.021-0.064 ppm in foliage, 0.134-0.220 ppm in roots, 0.076-0.263 ppm in hulls, and 0.045-0.109 ppm in seed harvested 120 days (at maturity) following a single application of [^{14}C]glufosinate-ammonium at 0.67 lb ai/A (0.8x the maximum proposed seasonal rate). Samples of canola commodities were also collected at shorter PTIs; TRR were 144.578 ppm in an entire plant collected at a 1-hour PTI and were 3.207 and 5.343 ppm in foliage, and 3.807 and 5.192 ppm in roots collected at a 21-day PTI.

2c. In mature canola seed and hulls, ~40-58% of TRR was identified. Glufosinate-ammonium and the MP-propionic acid metabolite were the major residues identified, accounting for 5.0-44.8% of TRR (0.007-0.118 ppm). The N-acetyl-glufosinate metabolite was a minor residue, accounting for 1.1-13.9% TRR (0.001-0.037 ppm). Radioactive residues associated with water-soluble polysaccharides and/or proteins accounted for 14.9% of TRR (0.016 ppm) in canola seed.

2d. In a canola plant harvested 1 hour posttreatment, the parent accounted for the majority of the radioactivity (72.9% TRR, 105.4 ppm); N-acetyl-glufosinate was identified at 18.2% of TRR (26.3 ppm). In foliage harvested 21 days posttreatment, the major residue was N-acetyl-glufosinate (60.2% TRR, 3.22 ppm); the parent was present at 20.7% of TRR (1.11 ppm) and a small amount of MP-propionic acid was identified (6.7% TRR, 0.358 ppm).

DETAILED CONSIDERATIONS

OPPTS GLN 860.1300: Nature of the Residue - Plants

Sugar Beets

AgrEvo has submitted data from a study (citation listed below) investigating the metabolism of [^{14}C]glufosinate-ammonium in transgenic sugar beets. The in-life and analytical phases of the study were conducted by Hoechst Schering AgrEvo GmbH (Frankfurt, Germany).

44358601 Allan, J. (1996) (Carbon-14)-Labelled Glufosinate-ammonium (Hoe 039866) Metabolism in Genetically Modified Sugar Beets (Beta vulgaris ssp vulgaris var altissima) After Two Applications of (carbon-14)-Glufosinate-Ammonium at a Rate of 600 g a.i./ha Each: Lab Project Number: N:\DORR\TITLEPGS\A58109.DOC: CM95/035. Unpublished study prepared by Hoechst Schering AgrEvo GmbH. 67 p.

[3,4-^{14}C]Glufosinate-ammonium (specific activity 52,413 dpm/µg, radiochemical purity 98.3%) was prepared in solution from the hydrochloride salt of glufosinate and then mixed with formulation blank and water. The test substance was applied to sugar beets as a foliar spray 35 and 57 days after planting at 600 g ai/ha (0.54 lb ai/A); the total application rate was 1.2 kg ai/ha (1.07 lb ai/A; 1x the proposed maximum seasonal rate). The plants were maintained in a greenhouse. Samples were taken 0, 8, and 15 days following the first application, 0 and 21 days following the second application, and at maturity (146 days following the second application). The plants were divided into leaves (tops) and beets (when formed) and the tops were rinsed with water. Samples, including leaf rinsates, were then stored frozen (~-20 C) until analysis.

Total radioactive residues (TRR)

Samples of sugar beet commodities were homogenized and total radioactive residues were determined by liquid scintillation counting (LSC) following combustion. The TRR in sugar beet commodities are presented in Table 1. The petitioner additionally determined TRR by summing the radioactivity in extracts and solids following extraction. These TRR values are also presented in Table 1. The petitioner used the summed TRR values for all calculations of percent TRR; the reported limit of quantitation (LOQ) is 0.0011 ppm.

Table 1. Total radioactive residues (TRR) in samples of sugar beet commodities treated with [^{14}C]glufosinate-ammonium at a total application rate of 1.07 lb ai/A (1x the maximum proposed seasonal rate).

Commodity	TRR, ppm [^{14}C]glufosinate-ammonium equivalents					
	0-day PTI [a]		21-day PTI		146-day PTI	
	Combustion [b]	Extraction [c]	Combustion	Extraction	Combustion	Extraction
Rinsate	(11.95)	11.95	(1.68)	1.68	(0.06)	0.06
Tops	8.30	8.14	9.62	10.58	2.02	1.99
Total	20.25	20.08	11.30	12.26	2.08	2.05
Roots	1.97	2.01	6.47	6.75	0.84	0.93

[a] PTI = Posttreatment interval.
[b] TRR determined by combustion of entire sample; rinsate values are from LSC determinations.
[c] TRR determined by summing radioactivity in extracts and solids remaining following extraction.

Extraction and hydrolysis of residues

Homogenized sugar beet commodity samples were subjected to extraction and hydrolysis procedures for residue characterization and identification. During the fractionation procedures, aliquots of extracts and nonextractable residues were analyzed for radioactivity by LSC or combustion/LSC. Methanol and/or sodium azide was added to all extracts to prevent microbial degradation of ^{14}C-residues.

Samples were extracted with water:methanol (10:90, v:v) and centrifuged. The supernatant was isolated and the extraction was repeated until greater than 95% of TRR had been extracted, or the extract contained less than 2% of TRR. Extracts were concentrated by rotary evaporation at 40-50 C and reserved for HPLC and/or TLC analysis.

The distribution of ^{14}C-activity in the extracts and hydrolysates of sugar beet commodities is presented in Table 2.

Characterization/identification of residues

Extracts and hydrolysates were analyzed by TLC and HPLC. HPLC analyses were used to characterize/identify components in sample extracts. HPLC analyses were conducted using a Spherisorb SAX (strong basic anion exchange) column and an isocratic mobile phase of phosphoric acid/potassium dihydrogen phosphate (5 mM, pH = 2) and methanol [90:10 (v:v) for System 1 and 30:70 (v:v) for System 2]. The petitioner claimed that the two different solvent systems separated the analytes by two different mechanisms: System 1 by ion-exchange chromatography and System 2 by adsorption chromatography. Radioactivity was detected and quantified using a radioactivity monitor. Metabolites were identified by comparison of retention times and/or cochromatography with the following reference standards: [^{14}C]glufosinate-ammonium, [^{14}C]glufosinate-hydrochloride, [3-^{14}C]3-methylphosphinico-propionic acid (MP-propionic acid), [2-^{14}C]2-methylphosphinico-acetic acid, disodium [3,4-^{14}C]L-2-acetamido-4-methylphosphinato-butyrate (disodium N-acetyl-glufosinate), disodium [3,4-^{14}C]2-hydroxy-4-methylphosphinato butyrate, and [4-^{14}C]4-methylphosphinato butanoic acid.

The petitioner attempted to conduct TLC analyses to confirm identifications of metabolites. However, matrix effects prevented good separation of metabolites; therefore, identification of metabolites by HPLC was confirmed by HPLC using a different system.

A summary of the characterized and identified ^{14}C-residues in sugar beet commodities is presented in Table 3. The chemical names and structures of glufosinate-ammonium and its identified metabolites in sugar beet commodities are presented in Figure 1.

The petitioner also extracted and analyzed crop samples collected after the first treatment but before the second treatment. The rinsates of plants collected 3 hours, 8 days, and 15 days following the first treatment consisted entirely of parent glufosinate-ammonium (40.5%, 18.8%, and 13.8% of total plant TRR, respectively). Isomeric separation (using HPLC with a Crompak CR column) demonstrated equal proportions of D and L isomers in the rinsates. In tops collected 3 hours after the first treatment, 45.1% of TRR was parent and 9.0% TRR was N-acetyl-glufosinate. In tops collected 15 days after the first treatment, 29.3% of TRR was parent and 48.6% of TRR was N-acetyl-glufosinate. Isomeric separation of the parent peak in 0-day PTI tops demonstrated equal proportions of the D and L isomers. However, by 15 days following treatment, the D isomer of the parent accounted for 25.2% of TRR and the L-isomer accounted for 3.3% of TRR, indicating that acetylation of glufosinate-ammonium in the plant occurs with the L isomer.

Table 2. Distribution and characterization radioactive residues in sugar beet commodities treated with [^{14}C]glufosinate-ammonium at 1.07 lb ai/A.

Fraction	% TRR	ppm	Characterization/Identification	
0-day PTI Tops (TRR = 20.08 ppm)				
Rinsate	59.50	11.95	HPLC analysis resolved: Glufosinate-ammonium 59.4% TRR	11.92 ppm
Water:methanol	39.47	7.93	HPLC analysis resolved: Glufosinate-ammonium 25.2% TRR MP-propionic acid 0.4% TRR N-acetyl-glufosinate 13.4% TRR	5.05 ppm 0.07 ppm 2.68 ppm
Nonextractable	1.03	0.21	Not further analyzed (N/A).	
0-day PTI Roots (TRR = 2.01 ppm)				
Water:methanol	97.39	1.95	HPLC analysis resolved: Glufosinate-ammonium 30.9% TRR MP-propionic acid 2.2% TRR N-acetyl-glufosinate 64.3% TRR	0.62 ppm 0.04 ppm 1.28 ppm
Nonextractable	2.61	0.05	N/A.	
21-day PTI Tops (TRR = 12.26 ppm)				
Rinsate	13.68	1.68	HPLC analysis resolved: Glufosinate-ammonium 13.7% TRR	1.68 ppm
Water:methanol	85.03	10.42	HPLC analysis resolved: Glufosinate-ammonium 28.1% TRR MP-propionic acid 1.1% TRR N-acetyl-glufosinate 55.2% TRR	3.44 ppm 0.13 ppm 6.77 ppm
Nonextractable	1.29	0.16	N/A.	
21-day PTI Roots (TRR = 6.75 ppm)				
Water:methanol	96.39	6.50	HPLC analysis resolved: Glufosinate-ammonium 30.6% TRR MP-propionic acid 2.0% TRR N-acetyl-glufosinate 63.3% TRR	2.07 ppm 0.14 ppm 4.27 ppm
Nonextractable	3.61	0.24	N/A.	
146-day PTI Tops (TRR = 2.05 ppm)				
Rinsate	3.01	0.06	HPLC analysis resolved: Glufosinate-ammonium 2.3% TRR MP-propionic acid 0.3% TRR N-acetyl-glufosinate 0.2% TRR 2-methylphosphinico-acetic acid).07% TRR 0.001 ppm Plus 1 unknown peak at 0.09% TRR (0.002 ppm)	0.05 ppm 0.006 ppm 0.005 ppm
Water:methanol	94.48	1.94	HPLC analysis resolved: Glufosinate-ammonium 24.0% TRR MP-propionic acid 2.7% TRR N-acetyl-glufosinate 66.9% TRR	0.49 ppm 0.055 ppm 1.37 ppm

Fraction	% TRR	ppm	Characterization/Identification
Nonextractable	2.51	0.05	N/A.
146-day PTI Roots (TRR = 0.93 ppm)			
Water:methanol	96.25	0.89	HPLC analysis resolved: Glufosinate-ammonium 19.1% TRR 0.18 ppm MP-propionic acid 6.0% TRR 0.055 ppm N-acetyl-glufosinate 67.9% TRR 0.63 ppm Plus 1 unknown peak at 3.1% TRR (0.03 ppm).
Nonextractable	3.75	0.03	N/A.

Table 3. Summary of radioactive residues characterized/identified in sugar beet commodities treated with [^{14}C]glufosinate-ammonium at 1.07 lb ai/A.

Fraction	0-Day PTI Tops (TRR = 20.08 ppm)		21-Day PTI Tops (TRR = 12.26 ppm)		146-Day PTI Tops (TRR = 2.05 ppm)		0-Day PTI Roots (TRR = 2.01 ppm)		21-Day PTI Roots (TRR = 6.75 ppm)		146-Day PTI Roots (TRR = 0.93 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified [a]												
Glufosinate-ammonium	84.6	16.97	41.8	5.12	26.3	0.54	30.9	0.62	30.6	2.07	19.1	0.18
MP-propionic acid	0.4	0.07	1.1	0.13	3.0	0.061	2.2	0.04	2.0	0.14	6.0	0.055
N-acetyl-glufosinate	13.4	2.68	55.2	6.77	67.1	1.38	64.3	1.28	63.3	4.27	67.9	0.63
2-methylphosphinico-acetic acid	--	--	--	--	0.07	0.001	--	--	--	--	--	--
Total identified	**98.4**	**19.72**	**98.1**	**12.02**	**96.5**	**1.98**	**97.4**	**1.94**	**95.9**	**6.48**	**93.0**	**0.87**
Unknown	--	--	--	--	0.09	0.002	--	--	--	--	3.1	0.03
Nonextractable	1.03	0.21	1.29	0.16	2.51	0.05	2.61	0.05	3.61	0.24	3.75	0.03

[a] See Figure 1 for chemical structures of identified metabolites.

Storage stability

Samples of sugar beet commodities were stored frozen prior to analysis. The petitioner stated that samples were extracted and analyzed immediately after collection except that 0-day PTI roots were stored over 30 days (exact storage interval not provided) prior to analysis.

Radiovalidation of analytical method

The petitioner provided radiovalidation for analytical method HRAV-5A. Samples of tops and roots were extracted with water (for 30 min at room temperature) and the extracts were filtered, concentrated by rotary evaporation, and analyzed by HPLC (System 1); we note that determination of residues in method HRAV-5A is by GC using a flame-photometric detector. The radiovalidation results are presented in Table 4. The radiovalidation data indicate that method HRAV-5A adequately recovers residues of glufosinate-ammonium, MP-propionic acid, and N-acetyl-glufosinate from sugar beet commodities. The petitioner noted that analyses of samples extracted using the analytical method procedures were conducted after the samples had been stored frozen for 3 months; therefore, the results presented in Table 4 also demonstrate that residues of glufosinate ammonium and its metabolites are stable in sugar beet tops and roots during storage for up to 3 months.

Table 4. Residues of glufosinate-ammonium and its metabolites in sugar beet commodities determined in the metabolism study and determined following extraction according to analytical method HRAV-5A.

Fraction	146-Day PTI Tops (TRR = 2.05 ppm)				146-Day PTI Roots (TRR = 0.93 ppm)			
	Metabolism method		Analytical method		Metabolism method		Analytical method	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Glufosinate-ammonium	26.3	0.54	27.4	0.56	19.1	0.18	21.3	0.20
MP-propionic acid	3.0	0.062	3.1	0.06	6.0	0.055	5.9	0.05
N-acetyl-glufosinate	67.1	1.38	67.5	1.37	67.9	0.63	70.6	0.66

Study summary

The qualitative nature of the residue in transgenic sugar beets is adequately understood. Total radioactive residues (TRR) were 2.05 ppm in tops and 0.93 ppm in roots harvested 146 days following two applications of [^{14}C]glufosinate-ammonium at 0.54 lb ai/A (total application rate of 1.07 lb ai/A; 1x the maximum proposed seasonal rate). Samples of sugar beet commodities were also collected at shorter PTIs; TRR were 20.08 ppm in tops and 2.01 ppm in roots collected at a 0-day PTI and were 12.26 ppm in tops and 6.75 ppm in roots collected at a 21-day PTI.

In sugar beet tops and roots, ~93-98% of TRR was identified. The N-acetyl-glufosinate metabolite was the major residue in all commodities at all PTIs, except tops at the 0-day PTI, accounting for 55.2-67.9% of TRR (0.63-6.77 ppm). Glufosinate-ammonium accounted for 84.6% of TRR (16.97 ppm) in 0-day PTI tops, and accounted for 19.1-41.8% of TRR (0.18-5.12 ppm) in sugar beet commodities at other PTIs. 3-Methylphosphinico-propionic acid (MP-propionic acid) was identified at low levels in all sugar beet commodities at all PTIs (0.4-6.0% TRR, 0.04-0.14 ppm). One additional metabolite, 2-methylphosphinico-acetic acid, was identified in 146-DAT tops at 0.07% TRR (0.001 ppm).

Canola

AgrEvo has submitted data from a study (citations listed below) investigating the metabolism of [^{14}C]glufosinate-ammonium in transgenic canola. The in-life phase of the study was conducted by Research for Hire (Porterville, CA) and the analytical phase of the study was conducted by Hazleton Wisconsin, Inc. (Madison, WI).

44358606 Tshabalala, M. (1993) (Carbon-14)-Glufosinate-Ammonium: Nature of Seed Residue in Transgenic Canola (Rapeseed): Lab Project Number: HWI 6408-100: A51529: PM-039. Unpublished study prepared by Hazleton Wisconsin, Inc. 123 p.

44358607 Thalacker, F. (1994) (Carbon-14)-Glufosinate-Ammonium: Nature of the Residue in Transgenic Canola (Rapeseed): Lab Project Number: HWI 6408-100: A53141 Resulting from Multiple Number: HWI 6408-100: A53141: N:\DORR\TITLEPGS\A5141.DOC: Unpublished study prepared by Hazleton Wisconsin, Inc. 26 p.

[3,4-^{14}C]Glufosinate-ammonium (specific activity 20.62 mCi/g, radiochemical purity 98%) was prepared in solution from the hydrochloride salt of glufosinate and then mixed with nonlabeled glufosinate-ammonium, formulation blank, and water. The test substance was applied to canola plants at the 3- to 5-leaf stage as a foliar spray at 0.75 kg ai/ha (0.67 lb ai/A; 0.8x the proposed maximum seasonal rate). The plants were maintained in 52 pots in a greenhouse. An additional 10 pots containing canola plants served as controls and were not treated. One sample (whole plant) was collected 1 hour posttreatment. Samples were also collected 21 days posttreatment and at maturity, 120 days posttreatment. Immature plants were separated into top growth (foliage) and roots by cutting approximately 0.5-1 inch above the soil; roots were rinsed with water. Mature samples were separated into roots, foliage, and seed pods. The roots and foliage were separately rinsed with water (twice). The seed pods were rinsed with water (twice) and separated by hand into seeds and hulls. Samples, including rinsates, were then stored frozen (~-20 C) until analysis.

Total radioactive residues (TRR)

Samples of canola commodities were homogenized and total radioactive residues were determined by liquid scintillation counting (LSC) following combustion. At Research for Hire, radioactivity in rinsate samples was determined; however, radioactivity levels were not expressed in terms of radioactivity in the crop commodity. The limit of detection was 0.005 ppm. The TRR in canola commodities are presented in Table 5.

Table 5. Total radioactive residues (TRR) in samples of canola commodities treated with [^{14}C]glufosinate-ammonium at 0.67 lb ai/A (0.8x the maximum proposed seasonal rate).

Commodity	TRR, ppm [^{14}C]glufosinate-ammonium equivalents		
	1-hour PTI [a]	21-day PTI	120-day PTI
Whole plant	144.578	--	--
Rinse [b]	0.495	--	--
Foliage	--	3.207, 5.343	0.021, 0.024, 0.058, 0.064
Rinse [b]	--	0.050, 0.060	--
Roots	--	3.807, 5.192	0.134, 0.150, 0.187, 0.220
Hulls	--	--	0.076, 0.106, 0.125, 0.263
Seed	--	--	0.045, 0.054, 0.056, 0.109

[a] PTI = Posttreatment interval.
[b] Rinse of aluminum foil used to wrap sample after collection.

Extraction and hydrolysis of residues

Canola seed and hulls samples were subjected to extraction and hydrolysis procedures for residue characterization and identification. During the fractionation procedures, aliquots of extracts, hydrolysates, and nonextractable residues were analyzed for radioactivity by LSC or combustion/LSC. Sodium azide was added to all extracts to prevent microbial degradation of ^{14}C-residues.

Samples were extracted with hexane (three times) and acetone; extracts were isolated by centrifugation. Following acetone extraction, the remaining residues were freeze-dried and then extracted with water:methanol (90:10, v:v) and centrifuged.

Canola seed samples were subjected to further extraction procedures to characterize nonextractable residues. Residues were first subjected to a second extraction with water:methanol (90:10, v:v). Water-soluble polysaccharides and proteins were then extracted using 0.05 M dihydrogen potassium phosphate buffer (4 hours at room temperature). Lipids were extracted using methanol:chloroform (2:1, v:v) and then acetone. The remaining solids

were acid hydrolyzed using 1 M hydrochloric acid (at 55 C for 90 minutes) and then base hydrolyzed using 0.5 M sodium hydroxide (at 55 C for 45 minutes).

The canola plant that was collected 1 hour posttreatment as well as canola foliage collected 21 days posttreatment were extracted using the procedures of the enforcement analytical method. Samples were extracted with water and centrifuged; the extraction was repeated three more times and extracts were combined for HPLC analysis.

The distribution of ^{14}C-activity in the extracts and hydrolysates of canola commodities is presented in Table 6.

Characterization/identification of residues

Extracts and hydrolysates were analyzed by HPLC. HPLC analyses were conducted using either a Spherisorb SAX column and a gradient mobile phase of potassium dihydrogen phosphate buffer and methanol (System 1) or LC-8 and RX-C8 columns (in series) and an isocratic mobile phase of potassium dihydrogen phosphate buffer (System 2). Radioactivity was detected and quantified using fraction collection and LSC. Metabolites were identified by comparison of retention times and/or cochromatography with the following reference standards: [^{14}C]glufosinate-ammonium, [3-^{14}C]3-methylphosphinico-propionic acid (MP-propionic acid), disodium [3,4-^{14}C]L-2-acetamido-4-methylphosphinato-butyrate (disodium N-acetyl-glufosinate), and [2-^{14}C]2-methylphosphinico-acetic acid.

The petitioner provided the results of HPLC analyses using both System 1 and System 2. Different levels of the parent and the MP-propionic acid metabolite in extracts were observed depending on which system was used. No explanation was provided for this difference. Because the petitioner is proposing that both compounds be included in the tolerance expression, the results of both System 1 and System 2 analyses are presented in Table 6.

TLC analyses were conducted to confirm the identification of metabolites by HPLC. For seed and hull analyses, low levels of radioactivity and matrix effects prevented good separation of metabolites by TLC. The petitioner stated that analysis of the water:methanol extract of hulls was conducted to confirm the results of HPLC analysis of the seed extract. For plant and foliage extracts, TLC analyses were conducted on silica gel plates using a solvent system of isopropanol, acetic acid, and water. Radioactivity on TLC plates was detected and quantified using a signal analyzer and a digital autoradiography program. The presence of glufosinate-ammonium and N-acetyl-glufosinate in 1-hour PTI plant and 21-day PTI foliage extracts was confirmed by TLC.

The chemical names and structures of glufosinate-ammonium and its identified metabolites in canola commodities are presented in Figure 1.

Table 6. Distribution and characterization radioactive residues in canola commodities treated with [^{14}C]glufosinate-ammonium at 0.67 lb ai/A.

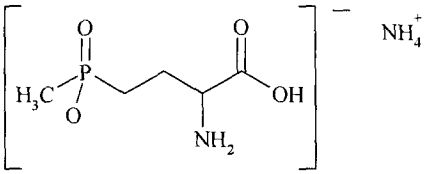
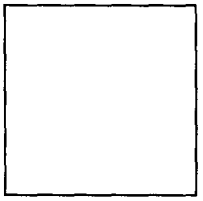
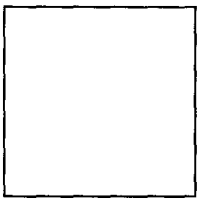
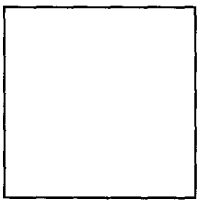
Fraction	% TRR [a]	ppm [a]	Characterization/Identification [a]
1-Hour PTI Plant (TRR = 144.58 ppm)			
Water	98.9	142.97	HPLC analysis (System 1) resolved: Glufosinate-ammonium 72.9% TRR 105.4 ppm N-acetyl-glufosinate 18.2% TRR 26.3 ppm Total identified 91.1% TRR 131.7 ppm
Nonextractable	0.24	0.34	Not further analyzed (N/A).
21-Day PTI Foliage (TRR = 5.343 ppm)			
Water	99.2	5.30	HPLC analysis (System 1) resolved: Glufosinate-ammonium 20.7% TRR 1.11 ppm MP-propionic acid 6.7% TRR 0.358 ppm N-acetyl-glufosinate 60.2% TRR 3.22 ppm Total identified 87.6% TRR 4.69 ppm
Nonextractable	2.24	0.12	N/A.
120-Day PTI Seeds (TRR = 0.109 ppm)			
Hexane	5.1	0.006	N/A.
Acetone	6.1	0.007	N/A.
Water:methanol	55.2	0.060	HPLC analysis (System 1) resolved: Glufosinate-ammonium 11.0% TRR 0.012 ppm MP-propionic acid 24.9% TRR 0.027 ppm N-acetyl-glufosinate 4.4% TRR 0.005 ppm Total identified 40.3% TRR 0.044 ppm HPLC analysis (System 2) resolved: Glufosinate-ammonium 30.9% TRR 0.034 ppm MP-propionic acid 6.6% TRR 0.007 ppm N-acetyl-glufosinate 1.1% TRR 0.001 ppm Total identified 38.7% TRR 0.042 ppm
Nonextractable	38.0	0.041	Subjected to sequential extraction/hydrolysis procedures using water:methanol, phosphate buffer, methanol:chloroform, acetone, mild acid, and mild base.
ater:methanol	4.1	0.004	N/A.
osphate	14.9	0.016	N/A.
thanol:chloroform	1.5	0.002	N/A.
etone	1.2	0.001	N/A.
id hydrolysate	5.5	0.006	N/A.
se hydrolysate	2.9	0.003	N/A.
nextractable	6.1	0.007	N/A.
120-Day PTI Hulls (TRR = 0.263 ppm)			
Hexane	ND [b]	ND	N/A.
Acetone	ND	ND	N/A.

Fraction	% TRR [a]	ppm [a]	Characterization/Identification [a]
Water:methanol	77.2	0.203	HPLC analysis (System 1) resolved: Glufosinate-ammonium 5.0% TRR 0.013 ppm MP-propionic acid 37.8% TRR 0.100 ppm N-acetyl-glufosinate 7.1% TRR 0.019 ppm Total identified 50.0% TRR 0.132 ppm HPLC analysis (System 2) resolved: MP-propionic acid 44.8% TRR 0.118 ppm N-acetyl-glufosinate 13.9% TRR 0.037 ppm Total identified 58.7% TRR 0.155 ppm Plus two unknowns at 23.2% and 2.3% TRR (0.061 and 0.006 ppm, respectively).
Nonextractable	37.2	0.098	N/A.

[a] Percent TRR and ppm values for extracts and metabolites were calculated by the study reviewer using percent-of-fraction values.

[b] ND = Not detected.

Figure 1. Glufosinate-ammonium and its metabolites in canola and sugar beet commodities.

Common Name Chemical Name	Structure	Substrate
Glufosinate-ammonium Ammonium-DL-homoalanin-4-yl(methyl) phosphinate		Canola foliage, seed, and hulls Sugar beet tops and roots
MP-propionic acid 3-Methylphosphinico-propionic acid		Canola foliage, seed, and hulls Sugar beet tops and roots
N-acetyl-glufosinate 2-Acetamido-4-methylphosphinico-butanoic acid		Canola foliage, seed, and hulls Sugar beet tops and roots
2-Methylphosphinico-acetic acid		Sugar beet tops

Storage stability

Samples of canola commodities were stored frozen prior to analysis. No dates of extraction or analysis were provided; however, the petitioner stated that canola hull and seed samples were extracted and analyzed within 6 months of collection. Samples of the whole plant and canola foliage were stored frozen at -70 C for up to 18 months prior to analysis (based on the dates of sample collection and study completion).

Because seed and hull analyses were completed within 6 months of collection, no supporting storage stability data are required for those commodities. Storage stability data for residues of glufosinate-ammonium, its MP-propionic acid metabolite, and N-acetyl-glufosinate in/on soybean forage and hay have been submitted previously (PP#5G4466 and PP#5F4578; CB Nos.

15081 and 16154, DP Barcodes D211531 and D219069, 3/7/96, M. Rodriguez). These data indicate that residues are stable during frozen storage for up to 12 months and are sufficient to support the storage conditions and intervals of samples of canola foliage in this study.

Study summary

The submitted study is marginally adequate to delineate the nature of the residue in transgenic canola. The test substance was applied at less than 1x the maximum proposed seasonal rate which resulted in low levels of radioactivity in canola seed, making identification of residues difficult. However, because adequate metabolism studies with transgenic field corn and soybeans have been submitted previously (PP#5G4466 and PP#5F4578; CB Nos. 15081 and 16154, DP Barcodes D211531 and D219069, 3/7/96, M. Rodriguez), no additional data pertaining to the metabolism of glufosinate-ammonium in canola will be required.

Total radioactive residues (TRR) were 0.021-0.064 ppm in foliage, 0.134-0.220 ppm in roots, 0.076-0.263 ppm in hulls, and 0.045-0.109 ppm in seed harvested 120 days (at maturity) following a single application of [^{14}C]glufosinate-ammonium at 0.67 lb ai/A (0.8x the maximum proposed seasonal rate). Samples of canola commodities were also collected at shorter PTIs; TRR were 144.578 ppm in an entire plant collected at a 1-hour PTI and were 3.207 and 5.343 ppm in foliage, and 3.807 and 5.192 ppm in roots collected at a 21-day PTI.

In mature canola seed and hulls, ~40-58% of TRR was identified. Glufosinate-ammonium and the MP-propionic acid metabolite were the major residues identified, accounting for 5.0-44.8% of TRR (0.007-0.118 ppm). The N-acetyl-glufosinate metabolite was a minor residue, accounting for 1.1-13.9% TRR (0.001-0.037 ppm). Radioactive residues associated with water-soluble polysaccharides and/or proteins accounted for 14.9% of TRR (0.016 ppm) in canola seed.

In a plant harvested 1 hour posttreatment, the parent accounted for the majority of the radioactivity (72.9% TRR, 105.4 ppm); N-acetyl-glufosinate was identified at 18.2% of TRR (26.3 ppm). In foliage harvested 21 days posttreatment, the major residue was N-acetyl-glufosinate (60.2% TRR, 3.22 ppm); the parent was present at 20.7% of TRR (1.11 ppm) and a small amount of MP-propionic acid was identified (6.7% TRR, 0.358 ppm).

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Chemical Name: Glufosinate

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